MAIL STOP AMENDMENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	Patent Application of Frederick H. Hausheer	Group Art Unit: 1614
Conf. No.:	3276	
Appln. No.	10/002,526	Examiner: Phyllis G. Spivack
Filed:	October 26, 2001	
For:	METHOD FOR TREATING PATIENTS FOR RADIATION EXPOSURE	Attorney Docket No. **066131-30US** (X-0211)

DECLARATION UNDER 37 C.F.R. § 1.132 OF STEPHEN T. SONIS, D.M.D., D.M.Sc.

I, Stephen T. Sonis, D.M.D., D.M.Sc., hereby declare:

1. I am Professor of Oral Medicine at the Harvard School of Dental Medicine and a member of the professional staff at Brigham and Women's Hospital and the Dana-Farber Cancer Institute. A copy of my Curriculum Vitae is attached to this Declaration.* As a result of my education, background and experience, I believe that I would be recognized as a person at least of ordinary skill in the art to which the present invention pertains, and am familiar with the level of skill of such persons in this art.

2. I was the principal investigator of a preclinical study (Study BNK-01) relating to the evaluation of dimesna (2,2′-dithiobis ethane sulfonic acid, disodium salt) to determine its utility in the reduction of oral mucositis induced by a single dose of radiation in Syrian golden hamsters. The study was conducted at a facility of Biomodels, L.L.C., in Cambridge, MA. At the time the study was conducted, I was not aware of the above-identified patent application. Although the study was funded by and the dimesna samples were provided

*Attached as Exhibit A

by BioNumerik Pharmaceuticals, Inc., which I understand, on information and belief, is the assignee of the above-identified application, and I have been in contact with Dr. Frederick H. Hausheer, the above-named Applicant and C.E.O. of BioNumerik Pharmaceuticals, Inc., there is no other relationship between me, The Harvard School of Dental Medicine, Brigham and Women's Hospital or Biomodels, L.L.C. and either Dr. Hausheer or BioNumerik Pharmaceuticals, Inc.

3. Oral ulcerative mucositis is a common, painful, dose-limiting toxicity of drug and radiation therapy for cancer, characterized by breakdown of the oral mucosa that results in the formulation of ulcerative lesions. Moderate to severe mucositis occurs in virtually all patients who receive radiation therapy for tumors of the head and neck and typically begins with cumulative exposures of 15 Gy and then worsens as total dosages of 60 Gy or more are reached. The Gray (Gy) is the International System of Units Measurement of absorbed dose radiation.

4. In connection with prior research unrelated to the present study, I developed an acute radiation model in Syrian Golden hamsters which has proven to be an accurate, efficient and cost-effective technique to provide a preliminary evaluation of anti-mucositis compounds (Sonis, ST, Tracey C. Shklar G., Jenson J. Florine D., "An animal Model for Mucositis Induced by Cancer Chemotherapy," *Oral Surg. Oral Med Oral Pathol* ***69(4)***:437-448 (1990)). The course of mucositis in this model is well defined and results in peak scores approximately 14-16 days following radiation. This model has been used to study specific mechanistic elements in the pathogenesis of mucositis.

5. In this study, an acute radiation dose of 40 Gy on day 0 was administered to sixteen test hamsters. Clinically significant mucositis was observed on days 12 through 28. The hamsters were divided into two groups of 8 each. Group 1 received saline and Group 2 received dimesna. Dosing was by intravenous infusion through a cannula surgically implanted in the jugular vein. The dose of dimesna was 3500 mg/kg delivered in a volume of 1.794 mL during a 15-minute infusion via jugular cannula. All animals received a dose of radiation of 40 Gy to the left buccal pouch on day 0. Mucositis was evaluated on day 6 and alternate days thereafter, through and including day 28. Mucositis scores in the control and treated groups were evaluated using established statistical methods to evaluate the potential efficacy of dimesna treatment for

the prevention or mitigation of radiation-induced oral mucositis. On each evaluation day, the number of animals with a blinded mucositis score of 3 or more in each drug treatment group was compared to the control group. Differences were compared on a cumulative basis and statistical significance was determined by chi-square analysis. Efficacy, in this analysis, is defined by a significant reduction in the number of days that a group of animals had ulcerations (scores of 3 or more) when compared to the control group. For each evaluation day, the scores of the control group were compared to those of the treated groups using non-parametric rank sum analysis. Treatment success in this experimental model was considered as a statistically significant lowering of scores in the treated group on two or more days from day 6 to day 28. All hamsters were weighed daily and their survival recorded to assess possible differences in animal weight among treatment groups as an indication for mucositis severity and/or possible toxicity resulting from the treatments. On each evaluation day, each animal was anesthetized by inhalation anesthesia (isofluorane) and the left buccal pouch was everted, photographed and scored for mucositis on a six-point scale that I developed in prior, unrelated studies. At the end of this study, each photograph was scored in a blinded fashion by two independent evaluators. The scores correspond to the following description, which depicts the conditions as shown in Figure 1, BNK-01, mucositis score scale, attached as Exhibit B:

Score:	Description:
0	Pouch completely healthy. No erythema or vasodilation.
1	Light to severe erythema and vasodilation. No erosion of mucosa.
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray color due to pseudomembrane. Cumulative size of ulcers should equal about ¼ of the pouch. Severe erythema and vasodilation.
4	Cumulative size of ulcers should equal about ½ of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth).

6. One hamster (hamster No. 1) in the group treated with dimesna died during the process of scoring and photographing the mucositis on day 10. The death was clearly due to

the anesthesia required for mucositis assessment and photography, and not the administration of dimesna.

7. The mean daily weight gains for each group revealed that the saline treated control Group 1 gained an average of 39.5% of their starting body weight by the end of the study. The dimesna treated Group 2 gained an average of 31.8% of their starting body weight by the end of the study. There was a statistically significant difference between the groups ($P = 0.034$), suggesting that the dimesna had a negative impact on weight gain at the dose of 3500 mg/kg.

8. In the saline control Group 1, the highest mucositis score was seen on days 14 – 18 when the mean mucositis score reached 2.75. In the dimesna treatment Group 2, the peak of mucositis was seen on day 18, with a mean score of 2.64. Thus, based on this score, it appeared that dimesna helped control radiation-induced mucositis. The significance of the differences in mucositis scores between the groups was evaluated using two statistical tests. In the first test, the number of days with a score of 3 or more was evaluated with a chi-squared test. In the second test, daily scores for each group were compared using a Mann-Whitney Rank Sum analysis. In the chi-squared analysis of animal days with the score of 3 or more, no statistically significant differences were seen ($P = 0.103$). However, the Mann-Whitney Rank Sum analysis showed a statistically significant improvement in mucositis in the dimesna treated group on day 14 ($P = 0.009$). Since the criteria for experimental significance for this study was set up to require statistical significance on two days, this does not represent a meaningful improvement.

9. In reviewing the mucositis scores, it became clear that the first hamster in the saline control group had not developed mucositis and had not in fact progressed beyond a score of 1. Since the overwhelming majority of hamsters that receive this dose of radiation usually reach a score of 3 for at least two days during the period of evaluation, this hamster represented an anomaly. Since the Syrian Golden hamsters are outbred, it is not surprising that the occasional hamster should be completely resistant to oral mucositis. It did make the analysis of the data more challenging and the data analysis was repeated with the data from hamster No. 1 excluded. In this analysis, the saline control Group 1 showed the highest mucositis score on days 14 – 18 when the mean mucositis score reached 3.0. In the group treated with dimesna, the peak

of mucositis was seen on day 18, with a mean score of 2.64. In the chi-squared analysis of animal days with a score of 3 or higher, statistically significant differences were seen ($P = 0.014$). The Mann-Whitney Rank Sum analysis also showed a statistically significant improvement in mucositis in the dimesna treated Group 2 on day 14 ($P < 0.001$). Based on the experimental design of this study, this improvement is not considered to be a meaningful improvement under the experimental significance criteria that requires statistical significance on two days.

10. Based upon the test results and analysis, it is my opinion that the mucositis data suggest that there may be some efficacy of dimesna at a 3500 mg/kg dosage in mitigating the radiation effects resulting in oral mucositis. However, the weight gain data suggests that 3500 mg/kg of dimesna may be having some toxic effect, which was not otherwise evaluated.

I hereby declare that all statements made herein of my own knowledge are true and that all statements on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application or any patent issuing thereon.

9/13/05
Date

Stephen T. Sonis, D.M.D., D.M.Sc.